



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07C 213/00, 217/58, 215/68, C07D 265/18	A1	(11) International Publication Number: WO 96/37457 (43) International Publication Date: 28 November 1996 (28.11.96)
(21) International Application Number: PCT/US96/07294 (22) International Filing Date: 21 May 1996 (21.05.96) (30) Priority Data: 08/450,330 25 May 1995 (25.05.95) US (71) Applicant: MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). (72) Inventors: THOMPSON, Andrew, S.; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). CORLEY, Edward, G.; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). GRABOWSKI, Edward, J., J.; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). YASUDA, Nobuyoshi; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).		(81) Designated States: AL, AM, AU, AZ, BB, BG, BR, BY, CA, CN, CZ, EE, GE, HU, IS, JP, KG, KR, KZ, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: ASYMMETRIC SYNTHESIS OF (-) 6-CHLORO-4-CYCLOPROPYL-ETHYNYL-4-TRIFLUOROMETHYL-1,4-DIHYDRO-2H-3,1-BENZOXAZIN-2-ONE		
(57) Abstract <p>An improved synthesis of a highly potent HIV reverse transcription inhibitor (I) is disclosed, involving an acetylide and a trifluoromethyl ketone which produces a chiral product in the presence of a chiral amino alcohol.</p> <div data-bbox="755 1165 1307 1438"><p style="text-align: right;">(I)</p></div>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgystan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

- 1 -

TITLE OF THE INVENTION

ASYMMETRIC SYNTHESIS OF (-) 6-CHLORO-4-CYCLOPROPYL-
ETHYNYL-4-TRIFLUOROMETHYL-1,4-DIHYDRO-2H-3,1-
BENZOXAZIN-2-ONE

5

BACKGROUND OF THE INVENTION

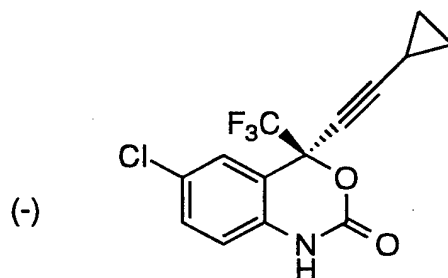
This case is related to Merck Case 18793IB which is a
continuation-in-part of 18793IA, which is a continuation-in-part of
Merck Case 18793, filed August 7, 1992, U.S.S.N. 07/926,607, and
10 19345.

A retrovirus designated human immunodeficiency virus
(HIV) is the etiological agent of the complex disease that includes
progressive destruction of the immune system (acquired immune
deficiency syndrome; AIDS) and degeneration of the central and
15 peripheral nervous system. This virus was previously known as LAV,
HTLV-III, or ARV. A common feature of retrovirus replication is
reverse transcription of the RNA genome by a virally encoded reverse
transcriptase to generate DNA copies of HIV sequences, a required step
in viral replication. It is known that some compounds are reverse
20 transcriptase inhibitors and are effective agents in the treatment of
AIDS and similar diseases, e.g., azidothymidine or AZT.

Nucleotide sequencing of HIV shows the presence of a pol
gene in one open reading frame [Ratner, L. *et al.*, *Nature*, 313, 277
(1985)]. Amino acid sequence homology provides evidence that the pol
25 sequence encodes reverse transcriptase, an endonuclease and an HIV
protease [Toh, H. *et al.*, *EMBO J.*, 4, 1267 (1985); Power, M.D. *et al.*,
Science, 231, 1567 (1986); Pearl, L.H. *et al.*, *Nature*, 329, 351 (1987)].

Applicants demonstrate a substantially improved synthesis
of an inhibitor of HIV reverse transcriptase, of the structure

- 2 -



named (-) 6-Chloro-4-cyclopropylethynyl-4-trifluoromethyl-1,4-dihydro-2H-3,1-benzoxazin-2-one, hereinafter "Compound A." This compound is highly potent, even against HIV reverse transcriptase resistant to other AIDS antiviral compounds.

Applicants have devised an asymmetric synthesis of Compound A. Prior methods required a racemate penultimate product, with a lower overall yield. The present invention relates to a direct synthesis of the optically active Compound A, by chiral addition to a ketone intermediate to give a tertiary alcohol, with an enantiomeric excess of greater than 95%.

Further, it is unexpected that reaction of an acetylide with a trifluoromethyl ketone produces an optically active product. In the present invention, this is achieved with a chiral amino alcohol to mediate the addition reaction along an asymmetric pathway.

Applicants have also discovered that heating (followed by cooling) the mixture of lithiated chiral amino alcohol and cyclopropylacetylene, before the addition of the trifluoroketone, boosts the enantiomeric excess from about 85% in a typical run to more than about 95%. The unusually high levels of optical activity (>95% ee) make this method advantageous and practical.

BRIEF DESCRIPTION OF THE INVENTION

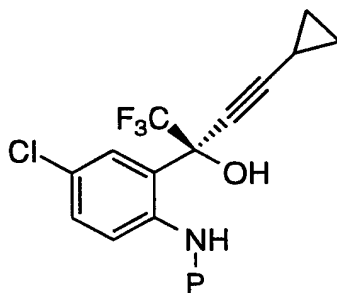
An improved synthesis of (-) 6-Chloro-4-cyclopropylethynyl-4-trifluoromethyl-1,4-dihydro-2H-3,1-benzoxazin-2-one is disclosed, and involves chiral addition to a ketone intermediate to give a tertiary alcohol. The compound is useful in the inhibition of HIV

- 3 -

reverse transcriptase (and its resistant varieties), the prevention of infection by HIV, the treatment of infection by HIV and in the treatment of AIDS and/or ARC, either as compounds, pharmaceutically acceptable salts (when appropriate), pharmaceutical composition ingredients, whether or not in combination with other antivirals, anti-infectives, immunomodulators, antibiotics or vaccines. Methods of treating AIDS, methods of preventing infection by HIV, and methods of treating infection by HIV are also disclosed.

10 DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS

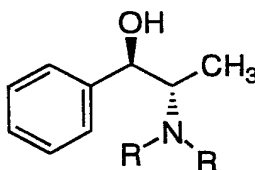
In this invention, there is disclosed a process for the asymmetric synthesis of the chiral compound of the structure



15

wherein P is an amino protecting group;
comprising the steps of:

- (a) providing a mixture of excess (1R,2S)-N-substituted norephedrine, of the structure



20

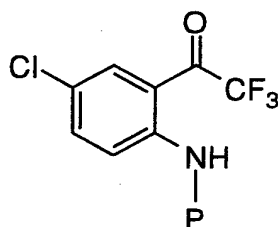
wherein R is C₁-4 alkyl, or -NR₂ forms pyrrolidinyl or piperidinyl;

- 4 -

with an excess of cyclopropylacetylene and an excess of a lithiating agent selected from n-butyllithium or sec-butyllithium or tert-butyllithium, at a temperature range of between about -78°C and about 10°C, in aprotic solvent;

5

- (b) mixing with the mixture of Step (a) about one equivalent of reactant of the structure

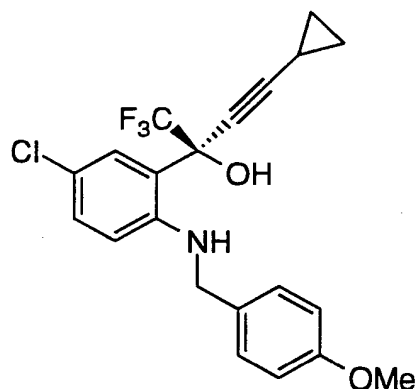


- 10 wherein P is an amino protecting group;
and maintaining the resulting reaction mixture at a temperature between about -78°C and about -20°C;

- 15 (c) quenching by adding a proton source;
(d) to give the desired compound.

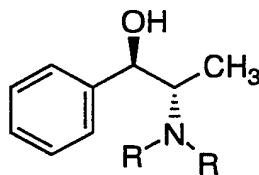
- 20 One embodiment of this invention is a process of the asymmetric synthesis of the chiral compound N-(4-methoxybenzyl)-6-chloro-2-[(R)-cyclo-propyl-ethynyl-hydroxy-trifluoromethyl]-methyl-aniline, of the structure

- 5 -



comprising the steps of:

- 5 (a) providing a mixture of excess (1R,2S)-N-substituted norephedrine, of the structure

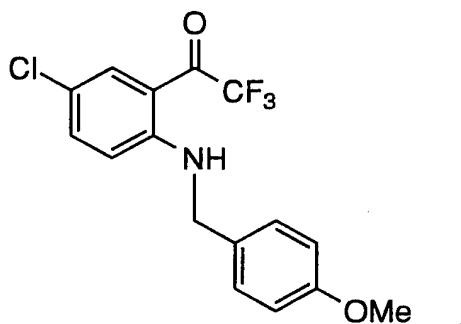


wherein R is C₁₋₄ alkyl, or -NR₂ forms pyrrolidinyl or piperidinyl;

- 10 with an excess of cyclopropylacetylene and an excess of a lithiating agent selected from n-butyllithium or sec-butyllithium or tert-butyllithium, at a temperature range between about -78°C and about 10°C, in aprotic solvent;

- 15 (b) mixing with the mixture of Step (a) about one equivalent of reactant N-(4-methoxybenzyl)-6-chloro-2-(2-trifluoro-1-oxo-ethyl)-aniline, of the structure

- 6 -



and maintaining the resulting reaction mixture at a temperature range of between about -78°C and about -20°C ;

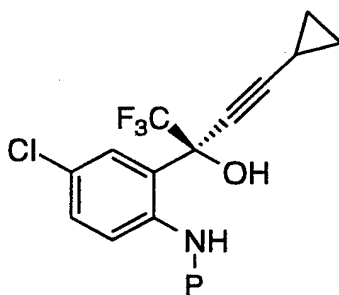
5

(c) quenching by adding a proton source;

(d) to give the desired compound.

10

Another embodiment of the same invention is a process for the asymmetric synthesis of the chiral compound of the structure

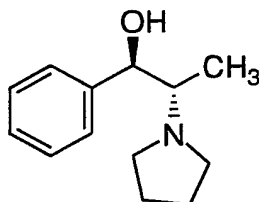


wherein P is an amino protecting group;

15 comprising the steps of:

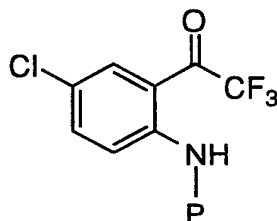
(a) providing a mixture of excess (1R,2S)-N-pyrrolidinyl norephedrine, of the structure

- 7 -



- with an excess of cyclopropylacetylene and an excess of a lithiating agent selected from n-butyllithium or sec-butyllithium or tert-butyllithium, at a temperature of about -15°C, in aprotic solvent;

- (b) mixing with the mixture of Step (a) about one equivalent of reactant of the structure



10

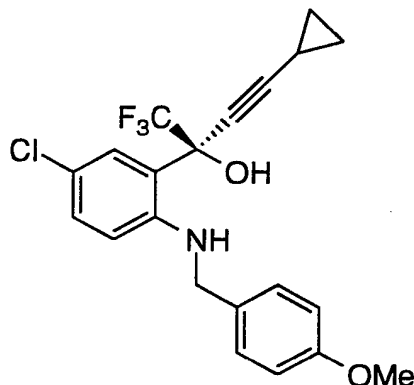
wherein P is an amino protecting group;
and maintaining the resulting reaction mixture at about -40°C;

- (c) quenching by adding a proton source;
- 15 (d) to give the desired compound.

Another embodiment of the same invention is a process for the asymmetric synthesis of the chiral compound N-(4-methoxybenzyl)-6-chloro-2-[(R)-cyclopropylethynyl-hydroxy-trifluoromethyl]-methyl-aniline, of the structure

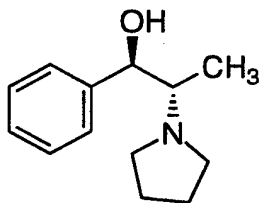
20

- 8 -



comprising the steps of:

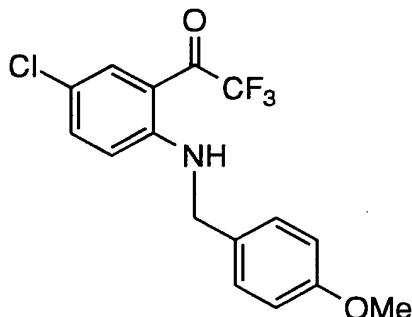
- 5 (a) providing a mixture of excess (1R,2S)-N-pyrrolidinylnorephedrine, of the structure



- 10 with an excess of cyclopropylacetylene and an excess of n-butyllithium, at a temperature of about -15°C, in aprotic solvent;

- (b) mixing with the mixture of Step (a) about one equivalent of reactant N-(4-methoxybenzyl)-6-chloro-2-(2-trifluoro-1-oxo-ethyl)-aniline, of the structure

- 9 -



and maintaining the resulting reaction mixture at about -40°C;

(c) quenching by adding a proton source;

5

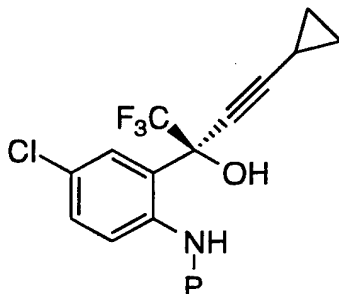
(d) to give the desired compound, in $\geq 85\%$ yield, $\geq 95\%$ ee.

Any of the above processes may be modified to improve enantiomeric excess with an additional heating (followed by cooling) step between step (a) and step (b), that is, the mixture of step (a) is heated to between about -10 °C and about 10 °C, for at least 5 minutes, then cooled to a temperature between about -78°C and about -20 °C, before the addition of the trifluoroketone. If the temperature of the mixture of step (a) already falls between about -10 °C and about 10 °C, raising the temperature may not improve the enantiomeric excess.

One preferred modification for improving enantiomeric excess is with an additional heating (followed by cooling) step between step (a) and step (b), that is, the mixture of step (a) is heated to between about -10 °C and about 0 °C, for between about 10 minutes and about 60 minutes, then cooled to a temperature of at least about -40°C, before the addition of the trifluoroketone.

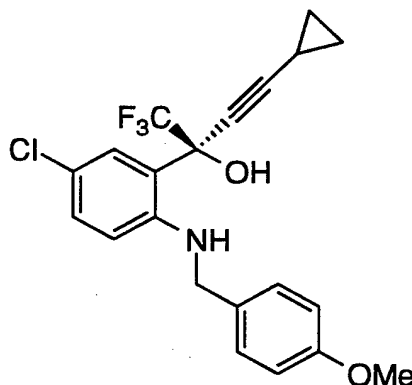
This invention also covers a compound of the structure

- 10 -



wherein P is an amino protecting group.

- 5 Another compound covered by this invention is N-(4-methoxybenzyl)-6-chloro-2-[(R)-cyclopropylethynyl-hydroxy-trifluoromethyl]-methyl-aniline, of the structure



- 10 At first, excess (1R,2S)-N-substituted norephedrine is mixed in aprotic solvent with excess cyclopropylacetylene, and the acidic protons are removed by lithiation with an excess of n-BuLi or sec-butyllithium or tert-butyllithium. The resulting mixture is maintained at a temperature range between about -78°C and about 10°C,
- 15 preferably at about -15°C.

If the resulting mixture is heated to between about -10°C and about 10°C, the product 6 is produced with substantially greater enantiomeric excess, typically about 95% instead of about 85% or less.

- 11 -

Some aspects of this invention eliminate this heating step, others include it.

In the processes of this present invention, P is any suitable amino protecting group, and includes, but is not limited to, benzyl
5 unsubstituted or substituted with C₁₋₄ alkyl; paramethoxy benzyl; para-nitrobenzyl; para-chlorobenzyl; 2,4-dichlorobenzyl; 2,4-dimethoxybenzyl; 4-methylsulfinyl benzyl; 9-anthrylmethyl; diphenylmethyl; or N-trialkylsilyl groups, according to T. W. Greene *et al.*, Protective groups in Organic Synthesis 2nd Ed. John Wiley 1991,
10 pp. 309-405. A preferable amino protecting group is paramethoxybenzyl. Once the reaction is initiated with the addition of about one equivalent of ketone 5, a trifluoroketone, the resulting reaction mixture is maintained between about -78°C and about -20°C, preferably about -40°C. The reaction is carried out in aprotic solvent or ethereal
15 solvent. Examples of aprotic solvents include THF, dioxane, Et₂O, benzene, DME, PhMe, n-octane, n-hexane, and cyclohexane, or mixtures thereof. One preferred solvent is THF. The incubation time of this reaction is at least 2-3 minutes after the addition of ketone.

At this point, the reaction mixture is quenched by addition
20 of a proton source in aqueous medium, typically a mild acid. Any such proton source is suitable, e.g., one preferred proton source is 1M citric acid. Another is 1M acetic acid.

The chiral product 6 is purified by conventional techniques.

The compounds of the present invention may have
25 asymmetric centers and may occur, except when specifically noted, as racemates, racemic mixtures or as individual diastereomers, or enantiomers, with all isomeric forms being included in the present invention. The term (+/-) is intended to encompass (+) optical isomers or (-) optical isomers or mixtures thereof.

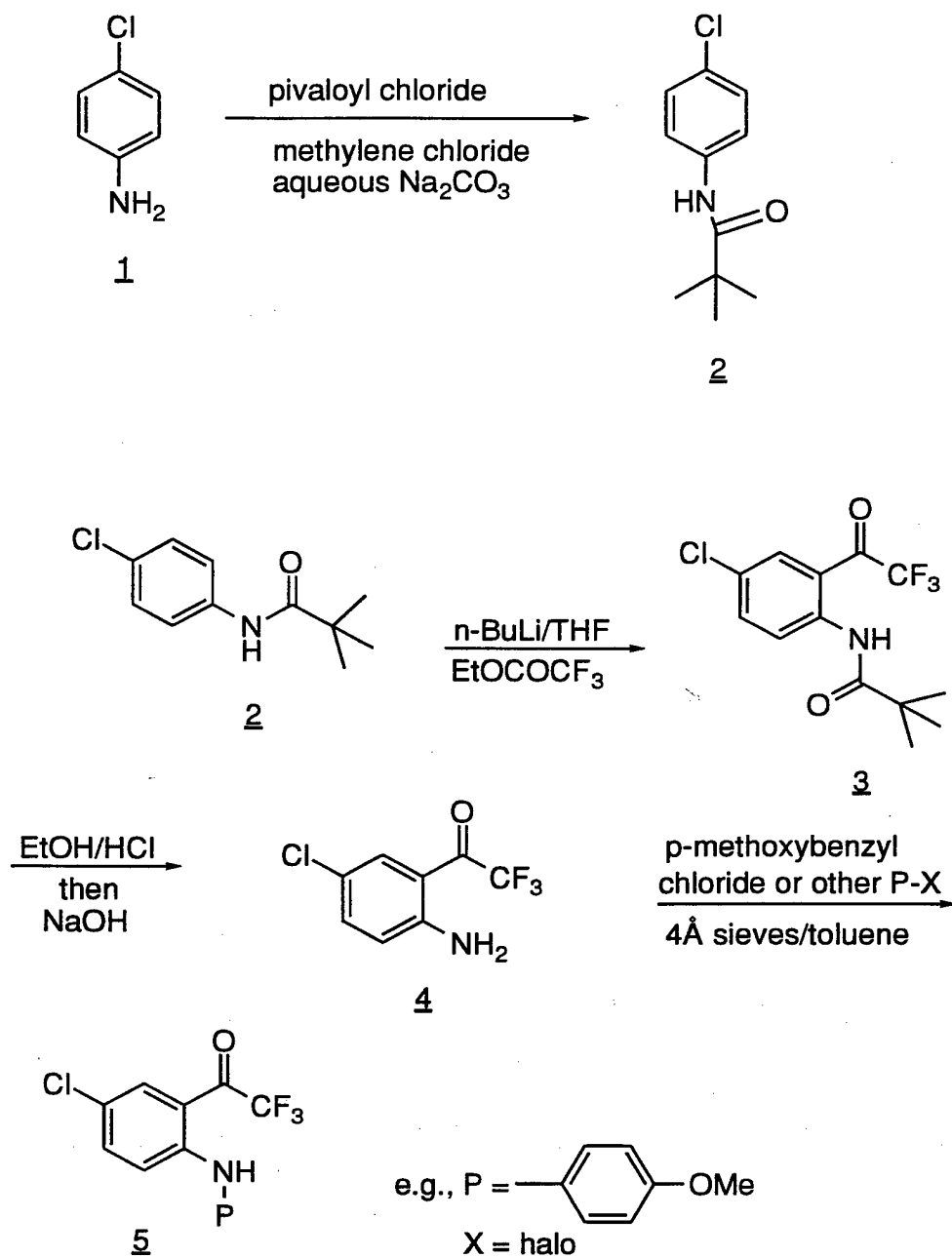
30 When any variable (e.g., R) occurs more than one time in any constituent or in Formula I, its definition on each occurrence is independent of its definition at every other occurrence. Also, combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

- 12 -

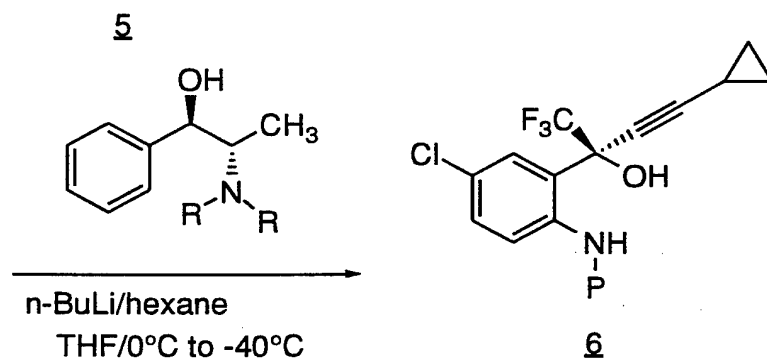
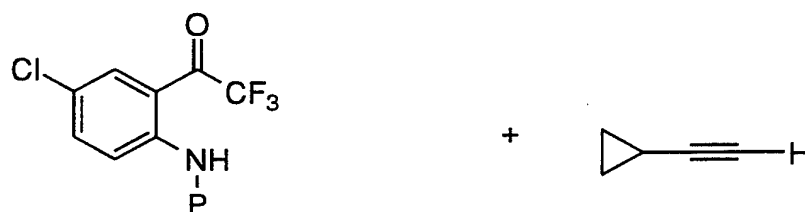
- As used herein except where noted, "alkyl" is intended to include both branched- and straight-chain saturated aliphatic hydrocarbon groups having the specified number of carbon atoms; if the number of carbon atoms is unspecified, "alkyl" is intended to
- 5 include 1 to 4 carbon atoms, both branched- and straight-chain saturated aliphatic hydrocarbon groups. "Halogen" or "halo" as used herein, means fluoro, chloro, bromo and iodo.

Compound A can be synthesized by the following method.

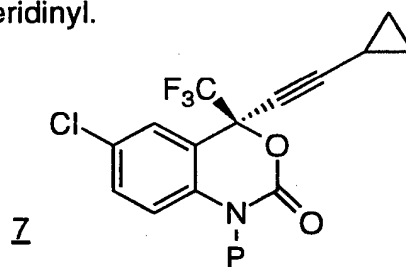
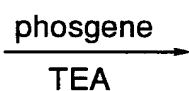
- 13 -

SCHEME I

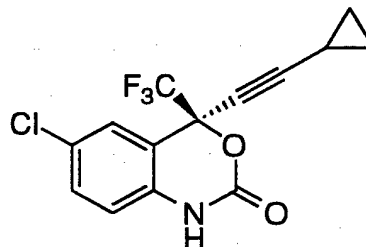
- 14 -

SCHEME I (CONT'D)

R is C_{1-4} alkyl, or $-\text{NR}_2$
may form pyrrolidiny or piperidiny.



(-)

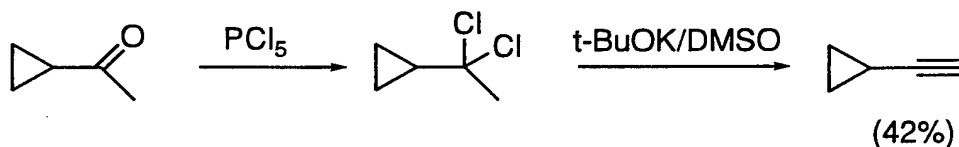


- 15 -

Cyclopropylacetylene, which is a reactant in the formation of 6, is prepared by the following alternative schemes;

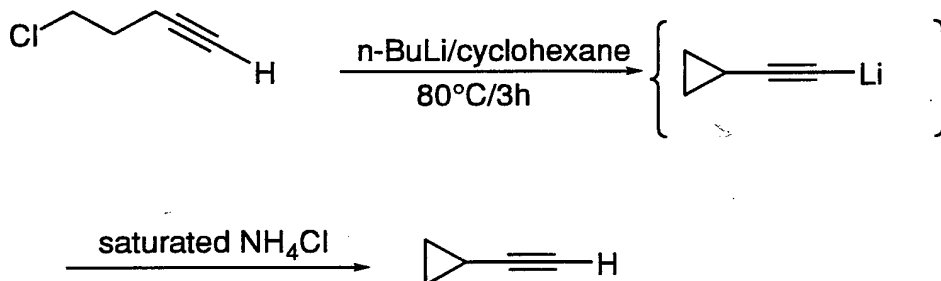
SCHEME IIA

5



as also described in C.E. Hudson *et al.*, *J. Am. Chem. Soc.*, 94, 1158 (1972) and W. Schoberth *et al.*, *Synthesis*, 703 (1972).

10

SCHEME IIB

Scheme IIB is illustrated in Example 3, and is preferred.

Compound A is useful in the preparation and execution of screening assays for antiviral compounds. For example, Compound A is useful for isolating enzyme mutants, which are excellent screening tools for more powerful antiviral compounds. Furthermore, Compound A is useful in establishing or determining the binding site of other antivirals to HIV reverse transcriptase, e.g., by competitive inhibition. Thus Compound A is a commercial product to be sold for these purposes.

Compound A is useful in the inhibition of HIV reverse transcriptase, the prevention or treatment of infection by human

- 16 -

immunodeficiency virus (HIV) and the treatment of consequent pathological conditions such as AIDS. Treating AIDS or preventing or treating infection by HIV is defined as including, but not limited to, treating a wide range of states of HIV infection: AIDS, ARC (AIDS
5 related complex), both symptomatic and asymptomatic, and actual or potential exposure to HIV. For example, Compound A is useful in treating infection by HIV after suspected past exposure to HIV by e.g., blood transfusion, exchange of body fluids, bites, accidental needle stick, or exposure to patient blood during surgery.

10 The particular advantage of Compound A is its potent inhibition against HIV reverse transcriptase rendered resistant to other antivirals, such as L-697,661, which is 3-([(4,7-dichloro-1,3-benzoxazol-2-yl)methyl]-amino)-5-ethyl-6-methyl-pyridin-2(1H)-one; or L-696,229, which is 3-[2-(1,3-benzoxazol-2-yl)ethyl]-5-ethyl-6-
15 methyl-pyridin-2(1H)-one; or AZT.

For these purposes, Compound A may be administered orally, parenterally (including subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques), by
inhalation spray, or rectally, in dosage unit formulations containing
20 conventional non-toxic pharmaceutically-acceptable carriers, adjuvants and vehicles.

Thus, in accordance with the present invention there is further provided a method of treating and a pharmaceutical composition for treating HIV infection and AIDS. The treatment involves
25 administering to a patient in need of such treatment a pharmaceutical composition comprising a pharmaceutical carrier and a therapeutically-effective amount of a compound of the present invention.

These pharmaceutical compositions may be in the form of orally-administrable suspensions or tablets; nasal sprays; sterile
30 injectable preparations, for example, as sterile injectable aqueous or oleagenous suspensions or suppositories.

When administered orally as a suspension, these compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may contain microcrystalline

- 17 -

cellulose for imparting bulk, alginic acid or sodium alginate as a suspending agent, methylcellulose as a viscosity enhancer, and sweeteners/flavoring agents known in the art. As immediate release tablets, these compositions may contain microcrystalline cellulose, dicalcium phosphate, starch, magnesium stearate and lactose and/or other excipients, binders, extenders, disintegrants, diluents and lubricants known in the art.

When administered by nasal aerosol or inhalation, these compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art.

The injectable solutions or suspensions may be formulated according to known art, using suitable non-toxic, parenterally-acceptable diluents or solvents, such as mannitol, 1,3-butanediol, water, Ringer's solution or isotonic sodium chloride solution, or suitable dispersing or wetting and suspending agents, such as sterile, bland, fixed oils, including synthetic mono- or diglycerides, and fatty acids, including oleic acid.

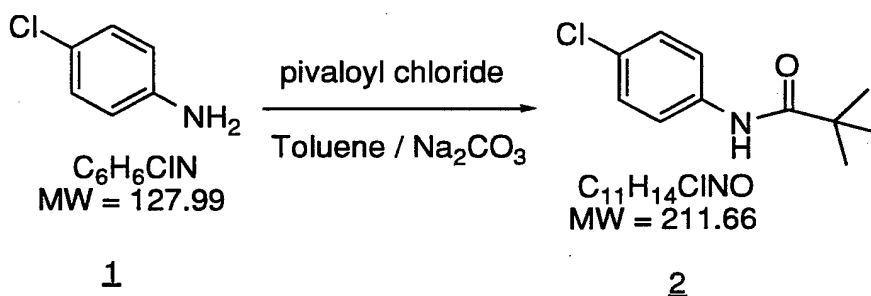
When rectally administered in the form of suppositories, these compositions may be prepared by mixing the drug with a suitable non-irritating excipient, such as cocoa butter, synthetic glyceride esters or polyethylene glycols, which are solid at ordinary temperatures, but liquidify and/or dissolve in the rectal cavity to release the drug.

Compound A can be administered orally to humans in a dosage range of 1 to 100 mg/kg body weight in divided doses. One preferred dosage range is 0.1 to 10 mg/kg body weight orally in divided doses. Another preferred dosage range is 0.1 to 20 mg/kg body weight orally in divided doses. For combination therapy with nucleoside analogs, a preferred dosage range is 0.1 to 20 mg/kg body weight for the compounds of this invention administered orally in divided doses, and 50 mg to 5 g/kg body weight for nucleoside analogs administered orally in divided doses. It will be understood, however, that the specific

dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the host undergoing therapy.

EXAMPLE 1

Preparation of 4-chlorophenyl-pivalamide



<u>Materials</u>	<u>Amt</u>	<u>MMole</u>	<u>MW</u>
4-chloroaniline	76 gm	596	127.57
Pivaloyl chloride (d=0.979)	74 ml	600	120.58
toluene	600 ml		
saturated Na ₂ CO ₃	95 ml		
D.I. water	225 ml		

15 To a solution of 4-chloroaniline (76 gm) in toluene (600 ml) was added saturated Na_2CO_3 (95 ml). The batch was cooled to 10°C and pivaloyl chloride (74 ml) was added dropwise over 45 minutes. The batch was stirred at $5\text{-}10^\circ\text{C}$ for 60 minutes while the progress of the reaction was monitored by HPLC.

20

Addition of pivaloyl chloride to the aniline was exothermic.

- 19 -

HPLC conditions: C-8 column, CH₃CN, water, phosphoric acid; gradient elution from 40:60:0.1 to 80:20 0.1 over 20 minutes, flow=1.0 ml/min, UV detection at 245 nm, starting material t_R=7.2 min, pivalamide t_R=12.6 min.

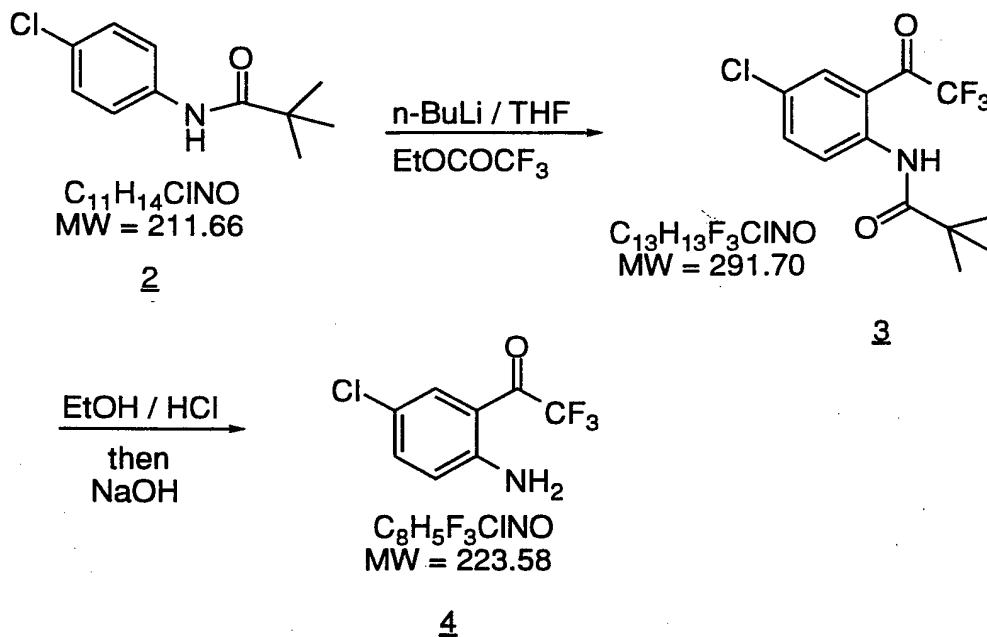
5

The product was isolated by filtration and washed with D.I. water (3 x 75 ml) and air dried under suction for 10 minutes. The product was dried in a vacuum oven at 40°C with an N₂ purge for 16h to afford 108.5 gm of product as fine white needles (86%).

10

EXAMPLE 2

Preparation of 4-chloro-keto-aniline 4



- 20 -

<u>Materials</u>	<u>Amt</u>	<u>MMole</u>	<u>MW</u>
4-chlorophenyl-pivalamide	10 gm	47.2	211.69
n-BuLi/hexane (2.5M)	38 ml	95	
ethyl trifluoroacetate (d=1.194)	6.7 ml	56.6	142.08
THF	75 ml		
Ethanol	90 ml		
6N HCl	50 ml	240	
hexane	90 ml		
2 N NaOH	15 ml		
D.I. water	350 ml		

5 In a 500 ml 3 necked flask the pivalamide (10 gm) was dissolved in dry THF (75 ml) and the mixture was cooled to 0°C. To this solution was added n-BuLi/hexane (2.5M, 38 ml) dropwise while allowing the internal temperature to rise to +15°C. The batch was aged at 0°C for 2h.

10 Addition of the first equivalent of n-BuLi to the pivalamide was highly exothermic. The exotherm was controlled by the rate of addition.

15 To the resulting light yellow suspension was added neat ethyl trifluoroacetate (6.7 ml), while allowing the internal temperature to rise to +10°C. The progress of the reaction was monitored by HPLC.

20 HPLC conditions: C-8 column, CH₃CN, water, phosphoric acid; gradient elution from 40:60:0.1 to 80:20 0.1 over 20 minutes, flow=1.0 ml/min, UV detection at 245 nm, starting pivalamide t_R=12.6 min, keto-pivalamide t_R=11.6 min. There was typically 85A% product and 10-15A% unreacted pivalamide.

The reaction was quenched by adding 6N HCl (10 ml) and D.I. water (20 ml).

- 21 -

HPLC assay at this point showed 13.1 gm (90%) of product.

The solution was concentrated to ca. 50 ml *in vacuo*, and flushed with ethanol (50 ml) to remove hexane and THF. To the batch was added 6N
5 HCl (40 ml) and the mixture was heated to reflux (80°C) for 1h.

HPLC assay shows 85-90A% of the keto-aniline, 10A% unreacted pivalamide. Thus the acylated material undergoes hydrolysis while unreacted pivalamide remains unchanged. The assay yield at this point
10 was 7.78 gm (74%).

The batch was concentrated to ca. 50 ml *in vacuo*, at which time a precipitate formed (presumably the HCl salt of the product). The distillation was discontinued and the batch was cooled to 0°C. After a
15 1h age, the batch was filtered and washed with hexane (3 x 30 ml).

The hexane washes remove unreacted pivalamide from the product. The solid is checked by HPLC to ensure it has been completely removed at this point. The filtrate and washes typically contain 1.2-1.5 gm of
20 product (8-12%). The majority of product loss was in the aqueous filtrate.

The salt was dried in a vacuum oven at 40°C for 16h to afford 10.4 gm of a solid which was 71.4% pure by weight (70% yield). The salt was
25 slurried in D.I. water (260 ml) and neutralized to a pH of ca. 6-7 with 2N NaOH (15 ml).

It was critical not to bring the pH above 9.0 due to product decomposition.

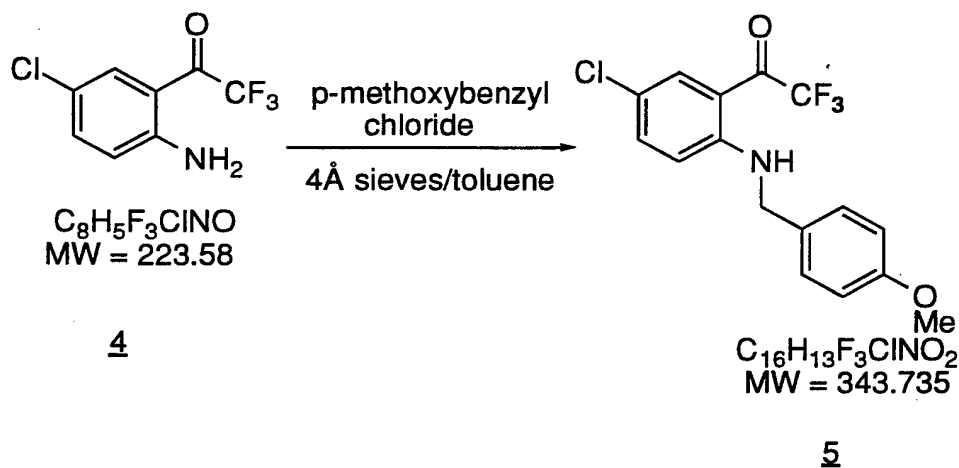
30 The resulting bright yellow solid was isolated by filtration and washed with D.I. water (2 x 25 ml). The product was dried in a vacuum oven at 40°C for 16h to afford 6 gm of keto aniline which was 96.6% pure by weight (54% yield).

- 22 -

The product is further purified by recrystallization from hexane.

EXAMPLE 3

5 Preparation of a N-4-methoxybenzyl-keto-aniline 5



<u>Materials</u>	<u>Amt</u>	<u>MMole</u>	<u>MW</u>
keto-aniline	15.5 gm	69.5	223.58
p-methoxy-benzylchloride	10.9 gm	69.8	
4Å molecular sieves	90 gm		
Toluene	70 ml		
Acetone	500 ml		
Hexane	120 ml		

10 In a 250 ml flask was charged the keto-aniline (15.5 gm), activated 4Å molecular sieves (50 gm) and toluene (75 ml). The mixture was stirred at 23°C under N₂ for 24h. Assay by HPLC showed ca. a 1:1 mixture of product and starting material.

15 HPLC conditions: C-8 column, CH₃CN, water, phosphoric acid; isocratic elution at 65:35:0.1 over 20 minutes, flow=1.0 ml/min, UV detection at 260 nm, toluene t_R=5.7 min, starting keto-aniline t_R=6.5 min, product t_R=15.0 min. There was typically 25A% of toluene.

- 23 -

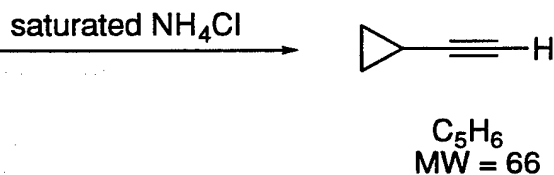
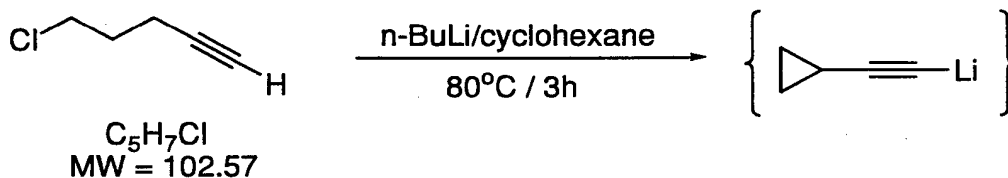
The reaction was charged with fresh molecular sieves (40 gm) and stirred for an additional 3 days at 23°C. The reaction was judged complete when less than 2A% of starting material remained.

Alternatively, basic alumina or silica gel may be used in place of sieves to remove HCl from the system.

The mixture was filtered through celite and washed with acetone (7 x 75 ml) until most of the yellow color was washed from the celite. The filtrate was concentrated to afford 27 gm of a yellow-orange oil which solidified on standing. The solid was purified by dissolving it in hot hexanes (100 ml). The batch was cooled to rt, then to 0°C in an ice-H₂O bath. After a 1.5h age, the batch was filtered and washed with cold hexanes (2 x 10 ml). The batch was air dried with suction for 10 minutes, then dried in a vacuum oven at 40°C for 2h. This afforded 20.5 gm (86%) of a bright yellow powder.

EXAMPLE 4

Preparation of cyclopropylacetylene



- 24 -

<u>Materials</u>	<u>Amt</u>	<u>MMole</u>	<u>MW</u>
5-chloro-1-pentyne	10 gm	98	102.57
n-BuLi/cyclohexane (2.0M)	122 ml	244	
cyclohexane	80 ml		
saturated NH ₄ Cl	50 ml		

To a solution of 5-chloro-1-pentyne in cyclohexane (80 ml) at 0°C under N₂ was added n-butyllithium in cyclohexane (2.0M, 122 ml).

5 The mixture was heated to 75°C for 5h.

Addition of n-butyllithium to the alkyne was exothermic, the temperature was maintained below +5°C during these additions using an ice-H₂O bath.

10

The progress of the cyclization step was monitored by HPLC. The reaction was considered complete when the assay yield was >90%.

15 HPLC conditions: Phenyl column, CH₃CN, water, phosphoric acid; 50:50:0.1 isocratic elution for 20 minutes, flow=1.0 ml/min, UV detection at 195 nm, starting material t_R=7.5 min, cyclopropylacetylene t_R=6.0 min. The product has a response factor which was 20 times greater than the starting material.

20 Once the cyclization step was complete, the reaction was cooled to 0°C and quenched with saturated NH₄Cl.

Assay of the organic phase by HPLC showed 5.5 gm of cyclopropylacetylene (85% yield).

25

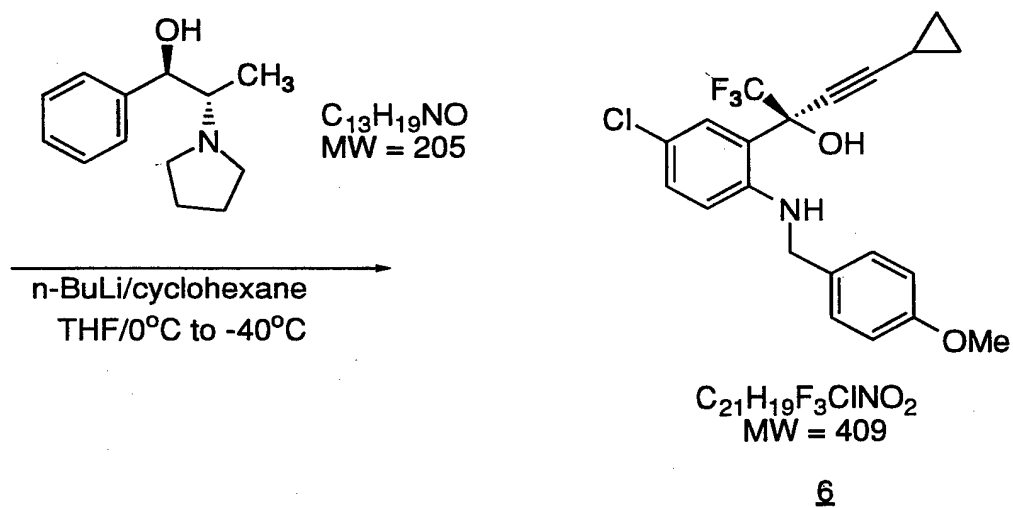
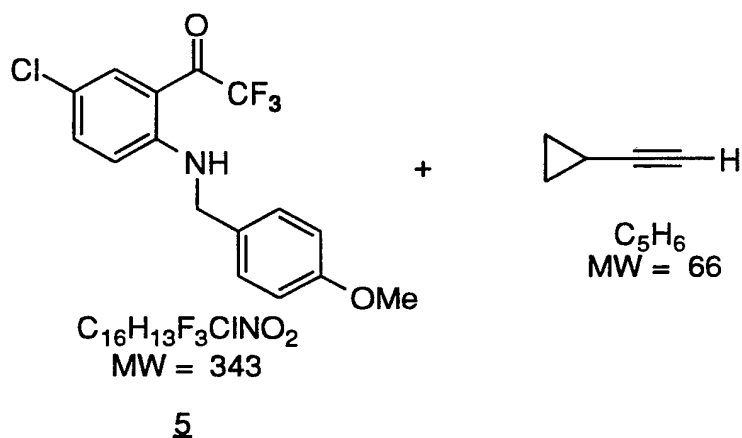
The product was purified by fractional distillation through a 6" x 0.5" column packed with 4 mm glass beads. The fraction with a boiling point between 45-75°C was collected.

- 25 -

This afforded 4.2 gm (65%) of cyclopropylacetylene as a colorless oil.

EXAMPLE 5

5 Preparation of the Amino Alcohol



- 26 -

<u>Materials</u>	<u>Amt</u>	<u>mmol</u>	<u>MW</u>
Ketone	10 gm	29.1	343
1R, 2S N-pyrrolidinyl norephedrine	13.1 gm	64	205
cyclopropyl acetylene (d=0.8)	5.3 mL	64	66
n-BuLi (2.6M in cyclohexane)	50 mL	125	
THF (KF = 20 µg/ml)	80 mL		
1M Citric acid	90 mL		
Ethyl acetate	75 mL		

The pyrrolidinyl ephedrine (13.1 gm) was dissolved in THF (55 mL) and the mixture was cooled to -15°C. To the mixture at -15°C under
5 N₂ was added neat cyclopropylacetylene (5.3 mL) and n-butyllithium (50 mL) dropwise. The mixture was aged at -5 to 0°C for 30 minutes, then cooled to -55°C.

10 The addition of n-butyllithium caused an exotherm which was maintained between -5 to 0°C by the rate of addition.

15 The ketone (10 gm) was dissolved in THF (25 mL) under N₂ and added to the anionic mixture over 15 minutes allowing the internal temperature to rise to -40°C during the addition. The resulting light orange solution was aged at -40°C for 60 minutes and quenched by
adding 1M citric acid (45 ml) and ethyl acetate (75 ml). The reaction was warmed to ambient temperature and the layers were separated. The organic layers were washed with 1M citric acid (45 mL). The reaction mixture was assayed by HPLC for percent conversion and product ee.

20 HPLC conditions: C-8 column, CH₃CN:water:phosphoric acid, isocratic elution 65:35:0.1 for 20 minutes, flow=1.0 ml/min, UV detection at 252 nm, st. material t_R=12.8 min, product t_R=10.3 min.

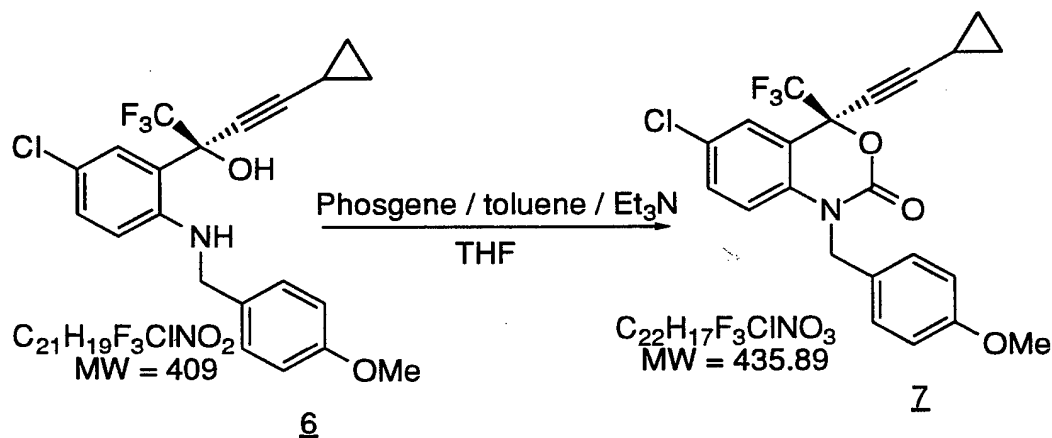
25 Chiral HPLC conditions: amylose stationary phase column, hexane:isopropanol 85:15 isocratic elution, flow=1.0 ml/min, UV

- 27 -

detection at 252 nm, st. material t_R =4.9 min, major enantiomer t_R =5.5 min, minor enantiomer t_R =25.0 min.

The enantiomeric excess was 96.5% and the reaction conversion was 93%, (6A% starting material). The assay yield was 85%. The product was purified by mixing in 10:1 heptane:toluene (65 mL) at 23°C for 18h. The product was isolated by filtration and oven dried at 35°C to afford 9.5 gm (80%) of product as a light yellow powder.

10

EXAMPLE 6Preparation of the BenzoxazinoneMaterials

	<u>Amt</u>	<u>mmol</u>	<u>MW</u>
amino alcohol	3.2 gm	7.8	409
Phosgene in toluene (1.93M)	4.6 ml	8.89	
triethylamine (d=0.726)	5.4 ml	39	101
THF (KF ≤ 100 µg/ml)	15 ml		
D.I. water	15 ml		
EtOAc	45 ml		
hexanes	30 ml		
1M citric acid	40 ml		
saturated brine	25 ml		

- 28 -

The amino-alcohol was dissolved in THF (15 ml) and cooled to -10°C under N₂. To the mixture was added triethylamine (5.4 ml) and phosgene in toluene (4.6 ml). The addition of phosgene caused an exotherm which was maintained below 20°C by the rate of addition.

- 5 The progress of the reaction was monitored by HPLC and was typically complete in 15 minutes.

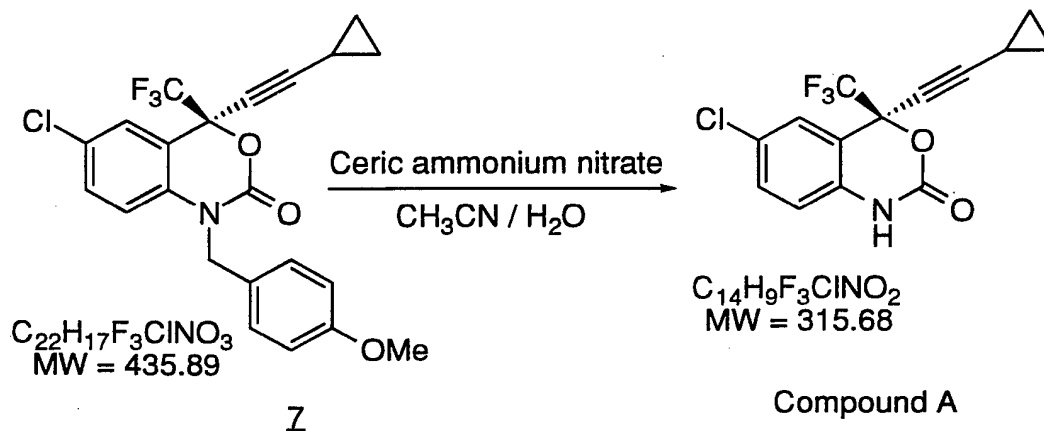
HPLC conditions: C-8 column, CH₃CN:water:phosphoric acid, gradient elution from 50:50:0.1 to 90:10:0.1 over 20 minutes, flow=1.5 ml/min, UV detection at 252 nm, st. material t_R=14.6 min, product t_R=16.0 min.

15 The reaction was cooled to 0°C and quenched with ice-cold water (15 ml) and ethyl acetate (20 ml). Saturated brine was used to break-up any emulsions. The organic layer was removed and the aqueous was extracted with ethyl acetate (15 ml). The combined organics were washed with 1M citric acid (40 ml) and saturated brine (25 ml). The organic was dried (Na₂SO₄) and concentrated *in vacuo* to afford 3.8 gm of a brown oil.

20 The product was crystallized from 5:1 hexane:ethyl acetate (25 ml), chilled to 0°C, aged for 1h and filtered. The cake was washed with cold 5:1 hexane:ethyl acetate (2 x 5 ml). The cake was air dried with suction to afford 2.9 gm (85%) of a light orange solid.

25

- 29 -

EXAMPLE 7Preparation of Compound A

5

<u>Materials</u>	<u>Amt</u>	<u>mmol</u>	<u>MW</u>
PMB Compound A, Z	0.8 gm	1.83	435
Ceric ammonium nitrate	4.4 gm	8.0	548.23
CH ₃ CN	15 ml		
ethyl acetate	30 ml		
D.I. water	30 ml		
saturated brine	10 ml		

The p-methoxybenzyl protected Compound A was dissolved in CH₃CN (15 ml). To this solution was added a solution of ceric ammonium nitrate (4.4 gm) in water (5 ml). The reaction was typically complete in 2h at 23°C as determined by HPLC.

10

HPLC conditions: C-8 column, CH₃CN:water:phosphoric acid, gradient elution from 50:50:0.1 to 90:10:0.1 over 20 minutes, flow=1.5 ml/min, UV detection at 252 nm, st. material *t*_R=16.0 min, product *t*_R=9.0 min.

15

The reaction was diluted with D.I. water (5 ml) and concentrated to ca. 1/2 volume. The product was extracted from the resulting aqueous

- 30 -

layer with ethyl acetate (2 x 15 ml). The combined organic was washed with D.I. water (2 x 10 ml) and brine (10 ml). The organic was concentrated *in vacuo* to afford a yellow gum. The product was isolated by silica gel chromatography.

5

REVERSE TRANSCRIPTASE ASSAY

The assay measures the incorporation of tritiated deoxyguanosine monophosphate by recombinant HIV reverse transcriptase (HIV RT_R) (or other RT) into acid-precipitable cDNA at the K_m values of dGTP and poly r(C)•oligo d(G)₁₂₋₁₈. The inhibitors of the present invention inhibit this incorporation.

The assays were carried out in 55 mM Tris (pH 8.2)-30 mM KCl-30 mM MgCl₂-1 mM dithiothreitol-20 µg of rC:dG₁₂₋₁₈ (Pharmacia) per ml-8 mM [3H]dGTP (New England Nuclear)-0.01% Triton X-100-50 mM ethylene glycol-bis(β-amino-ethyl ether)-N,N,N',N'-tetraacetic acid (EGTA)-1 mg of bovine serum albumin per ml. After 60 min of incubation at 37°C, acid-precipitable material was collected onto glass fiber filters by using a semiautomatic cell harvester. Bacterial cell extracts containing RT were diluted to within the linear range of the assay, and activity was determined in the presence and absence of inhibitor. Purified HIV-1 RT heterodimer produced in *E. coli* also served as a control. Results are determined as inhibitor concentration to give 50% inhibition (IC₅₀ wt), in nanomoles/liter. Compound A gave an IC₅₀ wt of 2 nM.

For the double mutant assay (dm), A17 RT was employed in the assay. A17 RT is resistant to various aminopyridones, as described in Nunberg, J.H. *et al.*, *J. Virol.*, 65, 4887 (1991). Results are measured as IC₅₀ dm in nanomoles/liter. Compound A gave an IC₅₀ dm of 85 nM.

30

- 31 -

CELL SPREAD ASSAY

Inhibition of the spread of HIV in cell culture was measured according to Nunberg, J. H. *et al.*, *J. Virol.*, 65, 4887 (1991).

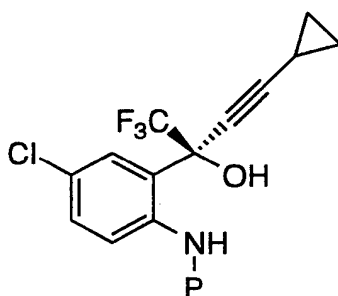
- 5 In this assay, MT-4 T-lymphoid cells were infected with HIV-1 (wild-type, unless otherwise indicated) by using a predetermined inoculum, and cultures were incubated for 24h. At this time, $\leq 1\%$ of the cells were positive by indirect immunofluorescence. Cells were then extensively washed and distributed into 96-well culture dishes. Serial
10 twofold dilutions of inhibitor were added to the wells, and cultures were continued for 3 additional days. At 4 days postinfection, 100% of the cells in control cultures were infected. HIV-1 p24 accumulation was directly correlated with virus spread. The cell culture inhibitory concentration was defined as the inhibitor concentration in
15 nanomoles/liter which reduced the spread of infection by at least 95%, or CIC95.

- While the foregoing specification teaches the principles of the present invention, with examples provided for the purpose of
20 illustration, it will be understood that the practice of the invention encompasses all of the usual variations, adaptations, or modifications, as come within the scope of the following claims and its equivalents.

- 32 -

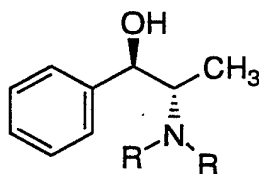
WHAT IS CLAIMED IS:

1. A process for the asymmetric synthesis of the chiral compound of the structure



wherein P is an amino protecting group;
comprising the steps of:

- (a) providing a mixture of excess (1R,2S)-N-substituted norephedrine, of the structure

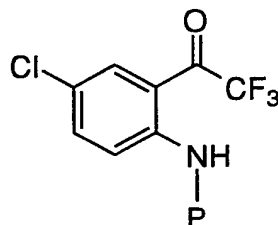


wherein R is C₁₋₄ alkyl, or -NR₂ forms pyrrolidinyl or piperidinyl;

- with an excess of cyclopropylacetylene and an excess of a lithiating agent selected from n-butyllithium or sec-butyllithium or tert-butyllithium, at a temperature of between about -78°C and about 10°C, in aprotic solvent;

- (b) mixing with the mixture of Step (a) about one equivalent of reactant of the structure

- 33 -



wherein P is an amino protecting group; and maintaining the resulting reaction mixture at a temperature between about -78°C and about -20°C;

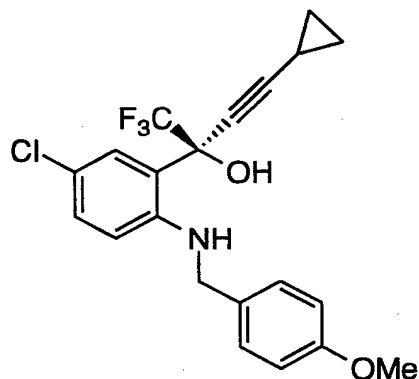
5

(c) quenching by adding a proton source;

(d) to give the desired compound.

10

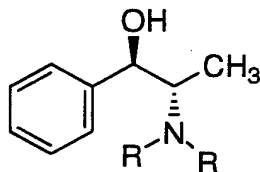
2. A process for the asymmetric synthesis of the chiral compound N-(4-methoxybenzyl)-6-chloro-2-[(R)-cyclopropylethynyl]-hydroxy-trifluoromethyl]-methyl-aniline, of the structure



15 comprising the steps of:

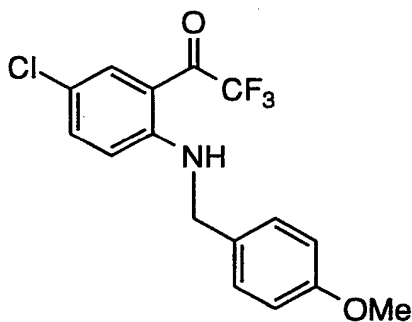
(a) providing a mixture of excess (1R,2S)-N-substituted norephedrine, of the structure

- 34 -



wherein R is C₁-4 alkyl, or -NR₂ forms pyrrolidinyl or piperidinyl;

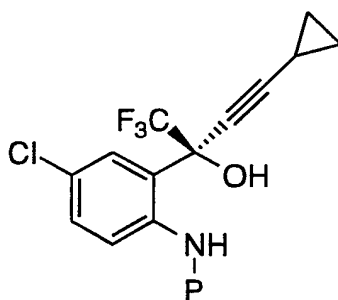
- 5 with an excess of cyclopropylacetylene and an excess of a lithiating agent selected from n-butyllithium, sec-butyllithium or tert-butyllithium, at a temperature of between about -78°C and about 10°C, in aprotic solvent;
- 10 (b) mixing with the mixture of Step (a) about one equivalent of reactant N-(4-methoxybenzyl)-6-chloro-2-(2-trifluoro-1-oxo-ethyl)-aniline, of the structure



- 15 and maintaining the resulting reaction mixture at a temperature between about -78°C and about -20°C;
- (c) quenching by adding a proton source;
- 20 (d) to give the desired compound.

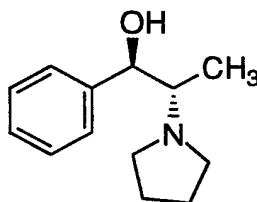
- 35 -

3. A process for the asymmetric synthesis of the chiral compound of the structure



5 wherein P is an amino protecting group; comprising the steps of:

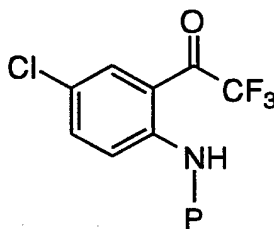
(a) providing a mixture of excess (1R,2S)-N-pyrrolidinyl norephedrine, of the structure



10

with an excess of cyclopropylacetylene and an excess of a lithiating agent selected from n-butyllithium or sec-butyllithium or tert-butyllithium, at a temperature of about -15°C, in aprotic solvent;

15 (b) mixing with the mixture of Step (a) about one equivalent of reactant of the structure

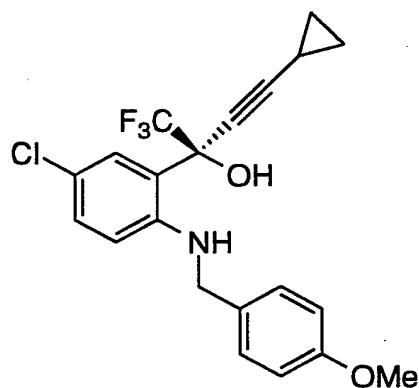


- 36 -

wherein P is an amino protecting group;
and maintaining the resulting reaction mixture at about -40°C;

- (c) quenching by adding a proton source;
5 (d) to give the desired compound.

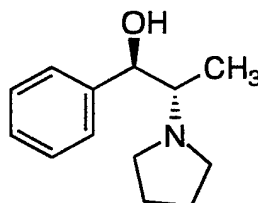
4. A process for the asymmetric synthesis of the chiral compound N-(4-methoxybenzyl)-6-chloro-2-[(R)-cyclopropylethynyl]-hydroxy-trifluoromethyl]-methyl-aniline, of the structure



10

comprising the steps of:

- (a) providing a mixture of excess (1R,2S)-N-pyrrolidinyl
15 norephedrine, of the structure

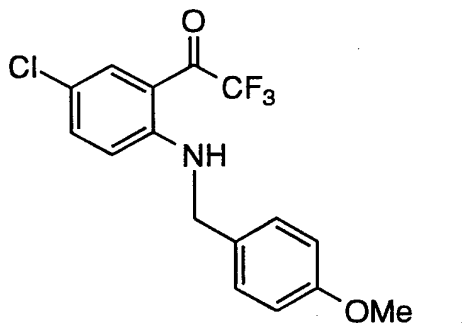


with an excess of cyclopropylacetylene and an excess of n-butyllithium,
at a temperature of about -15°C, in aprotic solvent;

20

- 37 -

- (b) mixing with the mixture of Step (a) about one equivalent of reactant N-(4-methoxybenzyl)-6-chloro-2-(2-trifluoro-1-oxo-ethyl)-aniline, of the structure



5

and maintaining the resulting reaction mixture at about -40°C;

- (c) quenching by adding a proton source;
- 10 (d) to give the desired compound, in $\geq 85\%$ yield, $\geq 95\%$ ee.

5. A process as in any of claims 1-2, wherein an additional heating step occurs between step (a) and step (b), that is, the mixture of step (a) is heated to between about -10 °C and about 10 °C, for at least 5 minutes, then cooled to a temperature between about -78°C and about -20 °C, before step (b).

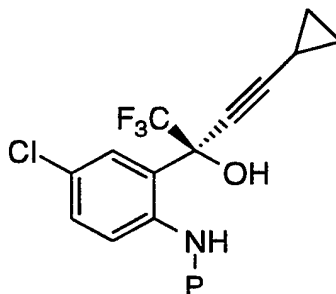
15

6. A process as in any of claims 3-4, wherein an additional heating step occurs between step (a) and step (b), that is, the mixture of step (a) is heated to between about -10 °C and about 0 °C, for between about 10 minutes and about 60 minutes, then cooled to a temperature of at least about -40°C, before step (b).

20

7. A compound of the structure

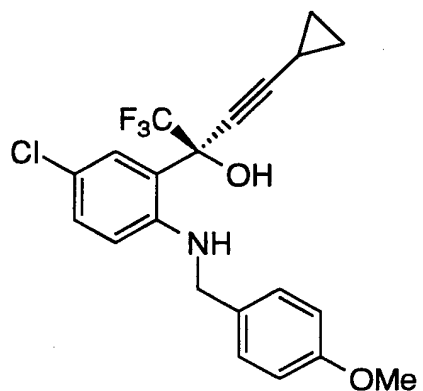
- 38 -



wherein P is an amino protecting group.

- 39 -

8. The compound N-(4-methoxybenzyl)-6-chloro-2-[(R)-cyclopropylethynyl-hydroxy-trifluoromethyl]-methyl-aniline, of the structure



INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/US 96/07294

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07C213/00 C07C217/58 C07C215/68 C07D265/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF ORGANIC CHEMISTRY, vol. 60, no. 6, 24 March 1995, EASTON US, pages 1590-94, XP002010918 M. A. HUFFMAN ET AL.: "Lithium alkoxides of cinchona alkaloids as chiral controllers for enantioselective acetylide addition to cyclic N-acetyl ketimines" see the whole document ---	1-6
A	DATABASE WPI Section Ch, Week 9212 Derwent Publications Ltd., London, GB; Class B05, AN 92-092946 XP002010923 & JP,A,04 036 252 (NIPPON ALKYL ALUMI) , 6 February 1992 see abstract --- -/-	1-6

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

20 August 1996

Date of mailing of the international search report

29.08.96

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Seufert, G

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/US 96/07294

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF ORGANIC CHEMISTRY, vol. 56, no. 13, 1991, EASTON US, pages 4264-68, XP002010919 K. SOAI ET AL.: "Chiral N,N-dialkylnorephedrine as catalysts of the highly enantioselective addition of the dialkylzincs to aliphatic and aromatic aldehydes. The asymmetric synthesis of secondary aliphatic and aromatic alcohols of high optical purity" see page 4265, left-hand column, last paragraph - page 4266, right-hand column, line 20; tables I,II ---	1-6
A	PATENT ABSTRACTS OF JAPAN vol. 005, no. 020 (C-042), 6 February 1981 & JP,A,55 145625 (MITSUBISHI CHEM IND LTD), 13 November 1980, see abstract ---	1-6
A	EP,A,0 582 455 (MERCK & CO INC) 9 February 1994 see page 18, scheme I, examples, especially example 6 ---	1-6
A	CHEMICAL ABSTRACTS, vol. 89, no. 13, 25 September 1978 Columbus, Ohio, US; abstract no. 108235, O. M. NEFEDOV ET AL.: "Synthesis and reaction of cyclopropylacetylene-type compounds" page 805; XP002010921 see abstract & IZV. AKAD. NAUK.SSSR, SER. KHIM., no. 6, 1978, pages 1339-44, ---	
A	DATABASE CROSSFIRE Beilstein Informationsgesellschaft GmbH XP002010922 see BRN 4765774 & J. ORG. CHEM. USSR, vol. 26, no. 10.2, 1990, pages 1902-04, ---	

	-/--	

INTERNATIONAL SEARCH REPORT

Intern: d Application No
PCT/US 96/07294

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>TETRAHEDRON LETTERS, vol. 36, no. 49, 1995, OXFORD GB, pages 8937-40, XP002010920 A. S. THOMPSON, ET AL.: "Use of ephedrine alkoxide to mediate enantioselective addition of an acetylde to a prochiral ketone: asymeric synthesis of th reverse transcriptase inhibitor L-743,726" see the whole document -----</p>	1-8

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 96/07294

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0582455	09-02-94	AU-B- 4449693	10-02-94
		BG-A- 99383	29-09-95
		CA-A- 2101572	08-02-94
		CN-A- 1090277	03-08-94
		CZ-A- 9500286	13-09-95
		FI-A- 950508	06-02-95
		HU-A- 71219	28-11-95
		JP-A- 6184124	05-07-94
		JP-B- 7080860	30-08-95
		NO-A- 950424	06-04-95
		NZ-A- 255216	28-05-96
		PL-A- 307348	15-05-95
		SI-A- 9300419	30-06-94
		SK-A- 16195	11-07-95
		WO-A- 9403440	17-02-94
		US-A- 5519021	21-05-96
		ZA-A- 9305724	03-03-94
